

THE MOBILITY OF [^{14}C]3-CHLORO-*p*-TOLUIDINE HYDROCHLORIDE IN A LOAM SOIL PROFILEKATHERINE C. IRWIN,[†] R. THOMAS PODOLL,[†] ROBERT I. STARR*[‡] and DONALD J. ELIAS[‡][†]SRI International, 333 Ravenswood Avenue, Menlo Park, California 94025, USA[‡]Denver Wildlife Research Center, U.S. Department of Agriculture, Denver, Colorado 80225-0266

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Abstract—The movement of radiolabeled 3-chloro-*p*-toluidine hydrochloride (CPTH) in a loam soil was evaluated in a column leaching study. Twenty-five-gram portions of the soil were treated with about 30 $\mu\text{g/g}$ of uniformly labeled [^{14}C]CPTH, and then incubated (aged) for 24 h under aerobic conditions; this soil was applied to untreated soil columns, which were then leached with dilute aqueous calcium sulfate for a total leachate volume equal to or greater than 50.8 times the cross-sectional area, or about 450 to 600 ml. Results indicated that about 2% of the initial radiolabeled compound leached through the soil profile, with the mean apparent partition coefficient calculated to be 65 ml/g. The soil columns were separated into 6-cm sections and extracted with dilute aqueous calcium sulfate, followed by 80% aqueous acetonitrile. About 90% of the [^{14}C]CPTH was sorbed to the uppermost 6 cm of soil. Following extraction, the residual soil was combusted, with these results indicating that an average 77% of the parent compound and products present in the upper 6 cm were bound to the soil colloids. Approximately 1% of the applied radiolabeled compound volatilized during the soil aging and leaching processes. The mean mass balance based upon [^{14}C] analyses was 102%. One major metabolite was detected in leachate fractions and soil extracts, which was tentatively identified as *N*-acetyl-3-chloro-*p*-toluidine, based upon HPLC retention times. This same degradation product was previously identified and confirmed using mass spectroscopy in CPTH aerobic soil biodegradation and fish accumulation studies. The extent to which CPTH was bound to the loam soil colloids, with only minimal movement through the soil profile, suggests a low potential for mobility of this pesticide in the soil environment.

Keywords—Leaching Mobility Pesticide CPTH Soil

INTRODUCTION

The compound 3-chloro-*p*-toluidine hydrochloride (CPTH) has been demonstrated to be an effective and unique pesticide because of its selective high toxicity to most pest bird species and low-to-moderate toxicity to the majority of predatory birds and mammals, and a lack of known secondary hazards when used in baits. The mode of action of CPTH in starlings has been suggested to be that of a nephrotoxin [1]. The acute toxicity (oral LD50) of the avicide to starlings is reported to be between 4 and 5 mg/kg of body weight [2]. Also, the primary metabolites of CPTH are indicated to be of low toxicity to birds and mammals [3].

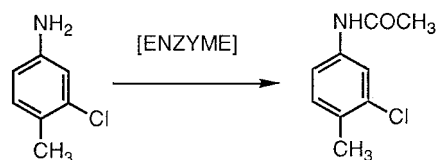
Under field conditions when the avicide is applied to soils as a seed or pellet bait to control various pest bird species (e.g., starlings or blackbirds in feedlots), some of the compound is undoubtedly solubilized and leached into the soil. However, limited data are available regarding the sorption and leaching of this minor-use pesticide in soil systems when used on a limited state-by-state basis. To provide additional environmental data and to comply with the U.S. Environmental Protection Agency (EPA) reregistration information requirements, a soil leaching study was conducted at our laboratory.

The aqueous solubility of CPTH has been reported to be about 92 mg/ml (S. Laut, 1990, unpublished data). Also, CPTH has an estimated pK_a of 3.9 for protonation of the amine group (S. Laut, 1990, unpublished data). Thus, in moist soils at pH values greater than 4.8, more than 90% of the chemical will be in the more volatile form as the free amine (3-chloro-*p*-toluidine

[CPT]; conversely, the ammonium ion form (CPTH) will be the major compound present only in acidic media below pH 3.

Bollag et al. [4] reported that the behavior of chloroanilines in soil was complex, due to competitive reactions involving both sorption and microbial degradation. Smith and Briggs [5] evaluated the persistence of the free aniline compound CPT in soil at concentrations that ranged from 2.5 to 50 $\mu\text{g/g}$; at the 72-h sampling point, the compound could not be detected.

Recent soil sorption research conducted at our laboratory using batch equilibrium methodology with five different soils that varied in organic carbon content (0.8–5.4%), pH (4.5–7.6), cation exchange capacity (13.4–25.3), and soil type indicated that equilibrium conditions could not be obtained during a 72-h equilibration period (K. Irwin and R.T. Podoll, 1994, unpublished data); soil sterilization was not used. Moreover, during HPLC analysis of the aqueous supernatant solutions from all soils, a peak was detected that corresponded (similar retention time) to the degradation product *N*-acetyl-3-chloro-*p*-toluidine, which had been identified previously in a CPTH aerobic soil biodegradation study [6]. Thus, due to degradation of the parent chemical during the 72-h equilibration period, equilibrium conditions could not be established, which prevented the calculation of sorption (K_d) values with any degree of accuracy. Structures of the parent compound and primary biodegradation product are illustrated below.



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Table 1. Chemical and physical properties of a Menlo Park loam soil

Texture	%				pH	CEC (meq/ 100 g)	H ₂ O at ½ bar %
	Sand	Silt	Clay	Organic carbon			
Loam	48	42	10	5.4	6.5	25.3	26.7

The objective of this study was to evaluate the potential for movement of CPTH in soils by estimating the leachability of radiolabeled CPTH and the primary metabolite through a loam soil profile as a function of water flow, following application of the chemical (aged for one half-life) to the soil.

MATERIALS AND METHODS

Test chemicals

Nonradiolabeled CPTH (Purina Mills, Bridgeton, MO, USA), purity of 99%, and uniformly ring-labeled [¹⁴C]CPTH (Sigma Chemical Company, St. Louis, MO, USA), specific activity of 2.25 mCi/mmol, were used; purity of the radiolabeled compound was >98%, as determined by HPLC. These chemicals were stored under dark conditions and refrigeration at about 4°C until used. The degradation product (*N*-acetyl-3-chloro-*p*-toluidine) was prepared at this laboratory; purity and compound identification were verified by melting point and mass spectrometry [7].

Soil selection and preparation

The loam soil was collected in Menlo Park, California, USA, within the grounds of SRI International. Following removal of approximately the upper 10 cm of surface soil, the material was passed through a 2-mm sieve to remove large soil particles and extraneous debris; this soil was also used in the CPTH aerobic soil biodegradation study [6]. Characterization data for the test soil are presented in Table 1.

Soil columns

The column leaching apparatus is illustrated in Fig. 1. Two segmented PVC columns (60 × 3.18-cm i.d.) were used in the study. Clear silicone sealant was used to join the individual column sections. Teflon® mesh screen (1 mm) was placed at the lower end of the column, and a 1.3-cm PVC ball valve was connected to the bottom of each column to allow flooding of the soil with the dilute aqueous CaSO₄ solution prior to leaching. In addition, a PVC T-connector was attached to the upper section of each column to allow removal of any volatiles that formed during the soil aging and leaching processes; using vacuum, volatile compounds present in the column headspace were passed through a series of traps containing ethylene glycol, 1 N H₂SO₄, and 1 N KOH, respectively. The leachate receivers were connected to a duplicate set of traps to collect volatiles (Fig. 1).

The columns were packed uniformly with the dry Menlo Park loam soil to a depth of about 45 cm. The bulk density of a packed column was determined from the soil weight divided by the volume of each column (Table 2). Each soil column was then saturated with 0.01 M CaSO₄ solution and weighed to determine the void volume. The columns were allowed to drain freely and then reweighed for calculation of a field moisture capacity.

Soil treatment and incubation (aging) procedures

Twenty-five-gram portions of the loam soil were weighed into 250-ml glass Erlenmeyer flasks that were fitted with ground glass stoppers. Deionized water was added to each sample to adjust the soil moisture to about 78% of field capacity. These samples were then equilibrated at ambient laboratory temperature for 3 d, after which the soils were uniformly surface-treated with 800 µl of [¹⁴C]CPTH (1,008 µg/ml) dissolved in 0.01 M CaSO₄; the soil was mixed thoroughly by stirring. This treatment resulted in about 32 ppm of CPTH (806 µg/25 g soil; 10.2 µCi), based upon uniform distribution of the chemical within the soil. The treated soil flasks were wrapped with aluminum foil to exclude light and incubated at ambient laboratory temperature (22 ± 3°C) for 24 h; this period was equivalent to one half-life for CPTH in the loam soil, as determined previously by Spangord et al. [6]. During the incubation or aging period, humidified air was passed over the soil samples (using a water aspirator) at rates that ranged from 50 to 70 ml/min; glass outlet tubes were connected in series to glass gas dispersion tubes containing ethylene glycol, 1 N H₂SO₄, or 1 N KOH. Upon completion of the soil incubation, these trapping solutions were stored at about -15°C until analyzed for [¹⁴C] activity.

Soil leaching procedures

The [¹⁴C]CPTH-treated aged soil samples were quantitatively transferred to columns containing about 45 cm of the untreated loam soil; total weight of soil was about 470 and 520 g for columns 1 and 2, respectively. A glass fiber filter disc (Gelman Filtration Products, Ann Arbor, MI, USA) was placed on top of the treated soil and a constant 12.7-cm head of the 0.01 M CaSO₄ eluent was then established. The ambient laboratory temperature during soil leaching was about 22 ± 5°C. The soil infiltration rates (cm/h) for the two soil columns are provided in Table 2.

The leachate fractions were analyzed immediately by HPLC, with an aliquot of each solution stored at about -15°C until analyzed by liquid scintillation counting (LSC). After a leachate volume equal to or greater than 50.8 times the column cross-sectional area was collected (450–600 ml), the soil columns were allowed to drain freely and then were separated into 6-cm sections (Fig. 1).

Analytical methods

Soil extraction procedures. Each soil section was weighed, transferred to a centrifuge tube, and then extracted with 0.01 M CaSO₄ using a mechanical shaker, followed by two extractions using 80% aqueous acetonitrile. The extraction mixture–soil suspension was centrifuged at 5,000 to 6,000 rpm for 30 min. The supernatant solution was decanted into a 50-ml graduated cylinder, the volume recorded, and a 1-ml aliquot then analyzed by HPLC; the solution remaining was stored at about -15°C until it could be analyzed by LSC.

The residual soil pellets (following centrifugation) were dried overnight under a fume hood and pulverized/homogenized using a stainless steel spatula. The dry soil samples were transferred to polyethylene bags, reweighed, and then stored at about -15°C until the bound parent chemical and metabolites could be oxidized to [¹⁴C]CO₂ and the resulting radioactivity determined by LSC.

Soil combustion and determination of radioactivity by LSC. Five 500-mg portions of each homogenized soil sample were combusted using a Model B306 Packard oxidizer (Downers

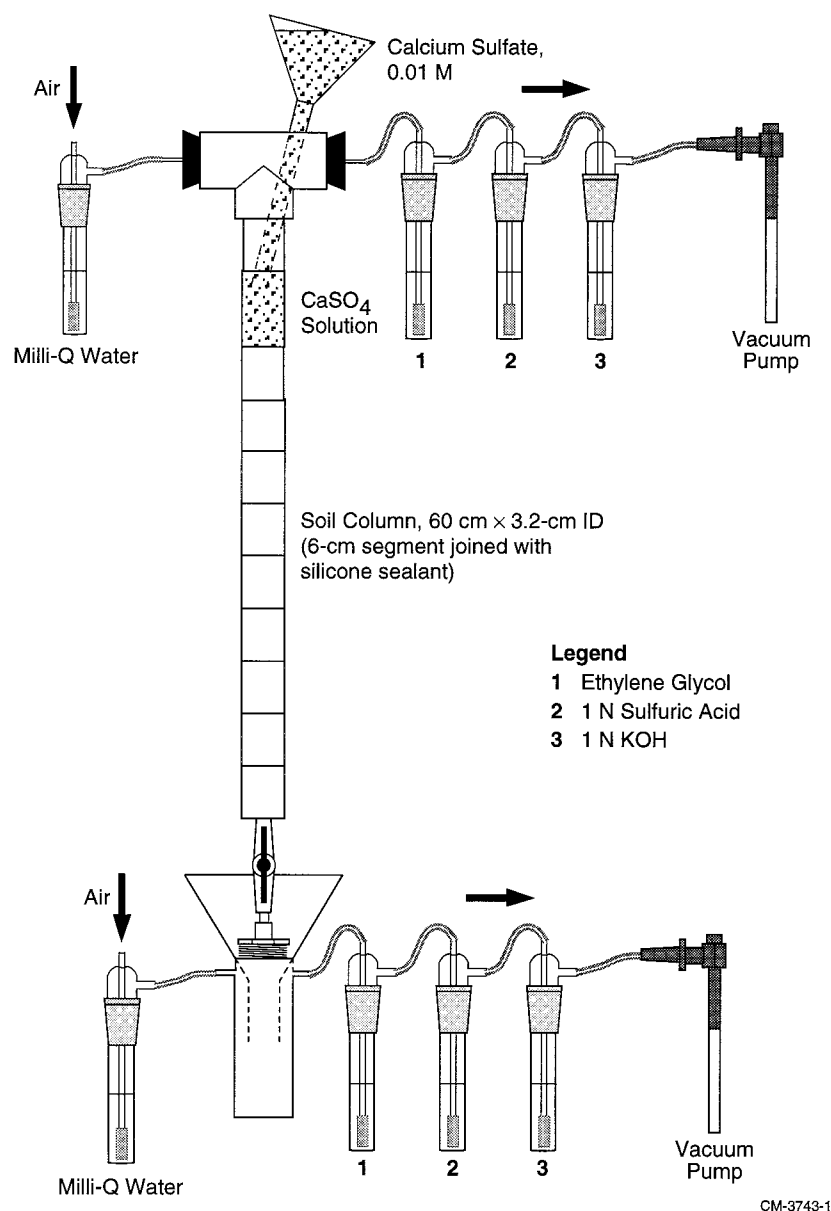


Fig. 1. Soil column leaching apparatus.

Table 2. Loam soil column specifications

Parameter	Soil column 1	Soil column 2
Column diameter (cm)	3.18	3.18
Total depth of soil (cm)	44.5	45.5
Bulk density (g/cm^3)	1.21	1.19
Void volume (ml)	190.8	188.7
Saturated moisture content (%)	34.3	32.8
Volume of leachate passed through column (ml)	445.6	599.8
Number of cross-sectional areas leached	56.4	75.6
Infiltration rate (cm/h)	10.6	6.1

Grove, IL, USA). Upon combustion, the radiolabeled CO_2 , trapped as a carbonate salt in 10 ml of Carbo-Sorb E[®], was flushed into scintillation vials that contained 14 ml of a scintillation cocktail Permafluor E+[®] (Packard, Meriden, CT, USA); each sample was counted for 2 min using the Beckman instrument described below. The counting efficiency for these analyses ranged from 77.3 to 81.5%.

LSC procedures. A Beckman Model LS 7000 liquid scintillation spectrometer was used to determine radioactivity in the column leachate fractions, extracts of the soil column segments, and column trap solutions. Five milliliters of Ecolite[®] (ICN, Irvine, CA, USA) was added to 0.2 ml of sample solution contained in duplicate for 2 min. The counting efficiency, deter-

Table 3. Movement of [^{14}C]3-chloro-*p*-toluidine hydrochloride (CPTH) in a loam soil profile

Leachate fraction	CPTH equivalents collected in leachate			
	Column 1		Column 2	
	μg	% of Applied ^a	μg	% of Applied ^a
1	0.10	0.01	1.05	0.13
2	0.05	0.01	0.74	0.09
3	1.08	0.13	1.44	0.18
4	3.86	0.48	3.15	0.39
5	3.12	0.39	5.72	0.71
6	0.88	0.11	1.80	0.22
7	4.15	0.51	6.59	0.82
8	0.72	0.09	—	—
Total	13.96	1.73	20.49	2.54

^a About 806 μg [^{14}C]CPTH applied.

mined by spiking samples with a [^{14}C] calibration standard, varied from 91.7 to 94.1%. Fifty microliters of 50% acetic acid was added to the alkaline KOH samples before analysis of radioactivity by LSC.

HPLC analytical parameters. The Hewlett-Packard 1090 operating conditions with UV detector were: column, C-18 reversed phase Lichrosphere 100 (Alltech Associates, Deerfield, IL, USA) (5 μm), 250 \times 4.6-mm i.d.; mobile phase, 45% acetonitrile:55% deionized water; flow rate, 1.0 ml/min; column temperature, 40°C; wavelength, 241 nm; injection volume, 50 or 100 μl ; CPTH retention time, 10.2 min; *N*-acetyl degradation product retention time, 7.5 min.

A RAMONA-92 (RAM) radiochemical detector (RAYTEST, Wilmington, DE, USA) was placed in series with the UV diode array detector. The retention time for CPTH using the RAM detector was 10.7 min, with that for the *N*-acetyl product 7.9 min.

A linear relationship was observed between chromatograph peak response and increasing concentrations of both CPTH and the *N*-acetyl degradation product between 0.16 and 39.5 $\mu\text{g}/\text{ml}$; these concentrations were determined (ChemStation Software) using linear regression of the calibration data for 50- μl injection volumes. Calibration standards were analyzed daily concurrently with the study samples. Detection limits for CPTH, determined for a signal-to-noise ratio of 2 with a 50- μl injection volume, were 0.07 $\mu\text{g}/\text{ml}$ with UV detection and 0.16 $\mu\text{g}/\text{ml}$ with RAM detection.

Table 4. Distribution of [^{14}C]3-chloro-*p*-toluidine hydrochloride (CPTH) in a loam soil profile after leaching

Soil section	Extractable [^{14}C]CPTH equivalents (μg)		Bound [^{14}C]CPTH equivalents (μg)		Extractable plus bound residues (%) ^a	
	Column 1	Column 2	Column 1	Column 2	Column 1	Column 2
1	140.4	66.9	539.0	701.5	84.3	95.3
2	9.9	6.6	31.5	21.6	5.1	3.5
3	4.1	1.0	20.6	25.3	3.1	3.3
4	1.4	0.6	4.3	4.4	0.7	0.6
5	0.5	0.9	1.6	1.3	0.3	0.3
6	0.5	0.5	1.2	1.4	0.2	0.2
7	0.8	0.6	1.1	1.2	0.2	0.2
8	0.4	0.6	1.3	1.5	0.2	0.3

^a About 806 μg [^{14}C]CPTH applied to each soil column.

Table 5. Distribution of [^{14}C]3-chloro-*p*-toluidine hydrochloride (CPTH) in a loam soil profile after leaching

	CPTH equivalents (μg)						% Recovery
	Leached	Ex-tracted	Bound	Recovered in traps		Total recovery	
				Upper	Lower		
Column 1	14.0	158.0	600.6	6.1	0.2	778.9	96.6
Column 2	20.5	77.7	758.2	4.6	0.3	861.3	106.9

^a About 806 μg [^{14}C]CPTH applied to soil columns.

Data analysis

A CA-Cricket Graph III[®] Program (Version 1.5, Computer Associates, Islandia, NY, USA) was used to plot the data, with a Microsoft Excel (Version 4.0, Redmond, WA, USA) software program used for calculating slopes, intercepts, and mean/standard deviation values.

RESULTS AND DISCUSSION

Movement of [^{14}C]CPTH and radiolabeled metabolites in a loam soil

Between 1.7 and 2.5% of the radiolabeled CPTH leached through the soil columns, which was directly proportional to the volume of leachate that passed through the loam soil (Tables 2 and 3). As illustrated in Table 4, the majority of CPTH applied to the soil was sorbed to the uppermost 6-cm sections of the two soil columns (84.3 and 95.3%). Moreover, only a small percentage (8.3 and 17.4%) of the CPTH that was sorbed to soil colloids in the top 6-cm soil segments was desorbed using a series of extraction solvents involving dilute aqueous CaSO_4 followed by aqueous acetonitrile. Thus, about 67 and 87% of the [^{14}C]CPTH and products were bound to soil colloids in the upper 6 cm of the soil in columns 1 and 2, respectively, and released only through combustion to [^{14}C]CO₂ (Table 4); these results, based upon total radiolabeled CO₂ recovered, did not differentiate between parent compound and products.

Between 0.6 and 0.8% of the initial CPTH that was applied to the moist and slightly acidic loam soil (pH = 6.5), and present as the free amine (CPT), volatilized during the 24-h soil aging and leaching periods and was collected in the column traps (Table 5); moreover, with both soil columns, the majority of radioactivity detected was contained primarily in the alkaline KOH trapping solutions. These figures (0.4–0.6%) compare favorably with that value obtained in the CPTH aerobic soil biodegradation study in which 0.6% of the initial CPTH application was recovered in the alkaline trapping solution after a 24-h incubation period [6].

The mass balance results (Table 5) illustrate that 97 and 107% of the initial [^{14}C] activity was recovered, based upon radioassays of leachate, soil extracts, soil sections, and traps of columns 1 and 2, respectively. Analysis of the leachate fractions and soil extract solutions by HPLC/UV confirmed the radiocarbon assays described above.

The only metabolite that was detected during the 24-h soil aging and leaching study was *N*-acetyl-3-chloro-*p*-toluidine (ACPTH). This radiolabeled product was found in acetonitrile extracts of the uppermost 6-cm soil sections of the two columns using HPLC with both UV (241 nm) and radiochemical (RAM) detection; approximately 0.9% of the applied [^{14}C]CPTH could be attributed to the radiolabeled *N*-acetyl product. Identification of this product was

based upon HPLC retention times. Retention times obtained for [^{14}C]CPTH and [^{14}C]ACPTH using HPLC with RAM detector were 10.7 and 7.9 min, respectively. This metabolite was identified in the CPTH aerobic soil biodegradation study [6].

The extent to which CPTH was sorbed to the soil colloids with only minimal movement through the soil profile suggests a low potential for the mobility of this pesticide in the soil environment.

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